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# A Convenient and Efficient Method for the Extraction and Fractionation of Steroid Hormones from Serum or Urine

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**Summary:** A convenient solid phase method for extracting and fractionating steroid hormones from serum or urine is presented. The influence of the solvent used for extraction, of the body fluid to be extracted, and of the degree of sample dilution on the efficiency of extraction was studied for progesterone and cortisol. The potency of fractionation was demonstrated by the separation of the steroid pairs, deoxycortisol – cortisol and oestradiol – oestriol, from a urine sample. Influence of ionic strength, pH and temperature on the reproducibility was assessed for deoxycortisol in undiluted urine. With respect to practicability and efficiency, this method has proved to be considerably superior to conventional liquid-liquid techniques.

*Eine praktische und effektive Methode für die Extraktion und Fraktionierung von Steroidhormonen aus Serum und Urin*

**Zusammenfassung:** Eine praktikable Festphasenmethode zur Extraktion und Fraktionierung von Steroidhormonen aus Serum und Urin wird beschrieben. Der Einfluß des Extraktionslösungsmittels, der Körperflüssigkeit selbst, und der Probenverdünnung auf die Extraktionsausbeute wird am Beispiel von Cortisol und Progesteron untersucht. Die Fraktionierungseigenschaften der Methode werden demonstriert am Beispiel der Trennung der Steroidpaare 11-Desoxycortisol und Cortisol sowie Östradiol und Östriol. Der Einfluß von Ionenstärke, pH und Temperatur auf die Reproduzierbarkeit des Fraktionierungsgrades wird für 11-Desoxycortisol in unverdünntem Urin untersucht. Hinsichtlich Praktikabilität und Effektivität erweist sich diese Methode den konventionellen Flüssigkeit-Flüssigkeitsmethoden deutlich überlegen.

## Introduction

Extraction of the analyte(s) from body fluids has hitherto been an essential prerequisite for the assessment of most steroid hormones. The classical techniques of liquid-liquid extraction are well known to be time consuming and troublesome, and subject to inconsistent and poor recovery. Furthermore, specific determination of single or multiple individual steroids often necessitates more or less extensive isolation prior to final quantitation (1).

Modifying an adsorption technique primarily designed for the extraction of drugs from urine (2), we evaluated a convenient method suitable both for extraction and fractionation of lipophilic steroid hormones from serum or urine, which has been applied in our laboratory for three years. In the present communication, the general applicability of this technique for steroid hormones is illustrated by the extraction and fractionation of some adrenal and gonadal steroids.

## Methods

### *Preparation of extraction tubes*

Paper discs are fixed onto the bottom of a plastic syringe by moistening them with a drop of water. The size of the plastic syringe depends on the volume of the sample to be extracted, e.g. a 50 ml syringe is used for samples of 10 ml. 0.6 g of dry porous Kieselguhr (Extrelut®, Fa. Merck, Darmstadt) per one ml of aqueous sample (serum or urine) is filled into the syringe using a measuring spoon. The powdery material is slightly compressed.

### *Extraction procedure*

For the study of extraction and fractionation potency, about 1.85 KBq of individual [$^3$H]steroids were added to the serum or urine sample. Samples were gently pipetted onto the extraction tube. After diffusion into the dry solid matrix for 10 min, extrac-

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tion is initiated by gently addition of organic solvent onto the Kieselguhr saturated by the aqueous sample. For fractionation purposes a sequence of solvents differing in polarity is added. Profiles of extraction were monitored by measuring $^3$H-radioactivity in 1.4 ml fractions using an LKB-collector (Type Ultrorac 7000). For routine use, the complete eluate is collected in conical glass tubes.

## Results

### *Extraction*

Progesterone and its trihydroxylated derivative, cortisol, were the labelled steroids chosen for evaluating the extraction characteristics of the present technique, because these steroids border approximately the range of polarity of the biologically relevant steroids. Influences of solvent, of the nature of the sample itself and the dilution of the sample on extraction efficiency is illustrated by the elution patterns of progesterone and cortisol in figure 1. In all these experiments, 2-ml-samples were studied. From pure serum, the more polar steroid cortisol is efficiently extracted by the more polar solvent, dichloromethane, but less by the less polar solvent diethyl ether (fig. 1a). The converse was found for the extractability of the less polar steroid progesterone (fig. 1b). From serum diluted 1:2 with water, both steroids were sufficiently extracted by reasonable amounts (20 ml) of both solvents studied (fig. 1c & 1d). From pure urine, both steroids are extracted with equal efficiency by both solvents studied (fig. 1e & 1f). The cumulative efficiency of extraction as studied in series analyses of eleven adrenal steroids processed in our laboratory (3) is more than 95%.

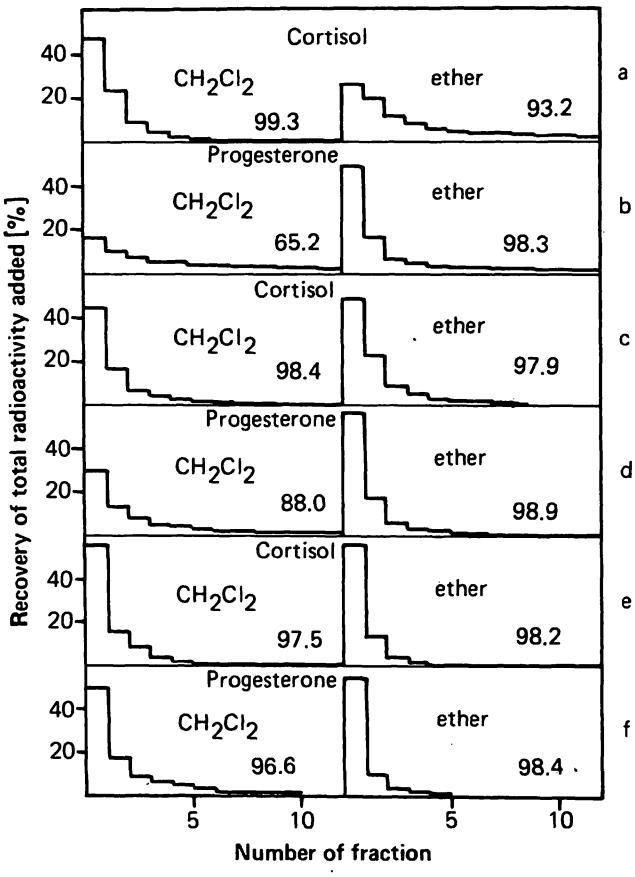


Fig. 1. Profiles of [$^3$H]progesterone and [$^3$H]cortisol eluted from 2 ml samples of serum and urine using diethyl ether and dichloromethane ($CH_2Cl_2$) as eluents. a and b: undiluted serum; c and d: serum diluted with one part of water; e and f: undiluted urine. The volume of each fraction was 1.4 ml. Values in the figures indicate percentage recovery of total radioactivity added.

### *Fractionation*

The resolving power of the present technique was studied for the separation of steroid pairs differing by one hydroxyl group, such as deoxycortisol-cortisol and oestradiol-oestriol. For these studies, 10 ml of undiluted urine were used. Several compositions of the solvent mixture carbon tetrachloride/dichloromethane were assessed for the optimal separation of these steroid pairs. The best conditions found and the corresponding elution profiles of the steroid pairs are outlined in figure 2, demonstrating an almost complete separation of these steroids. The statistical reproducibility of the degree of separation is documented in table 1 for 10 urine specimens.

While changes of ionic strength or pH had negligible influence on the degree of fractionation, a considerable influence of temperature was observed as illustrated in figure 3.

### *Practicability*

Including preparation of extraction tubes, one technician may handle about 40 samples in less than 40 minutes. Fractionation requires only a little more time. Since plastic syringes are reusable after cleaning, costs of the procedure are reduced to the relatively cheap kieselguhr, "Extrelut"®, and the solvents.

## Discussion

As already demonstrated for the problem of extracting drugs from urine (1, 4), the solid phase extraction technique also proves to be superior to the conventional liquid-liquid extraction for lipophilic steroid hormones (5, 6). Taking into account the points outlined under "Results", such as influence of material to be extracted, dilution of sample, solvent and temperature, the present technique provides several advantages:

1) the troublesome problem of emulsions inherent in liquid-liquid extraction is completely eliminated;

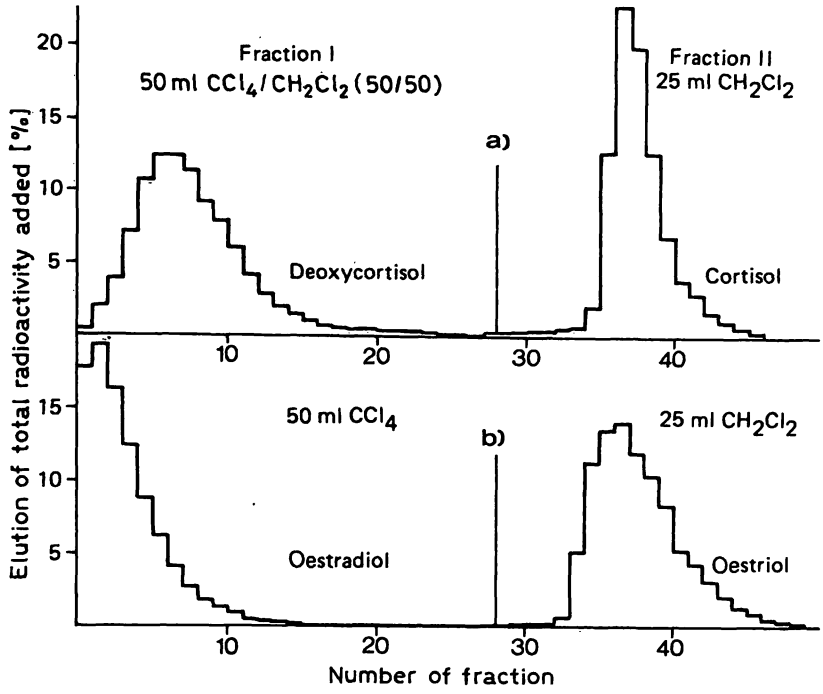


Fig. 2. Fractionation of steroid pairs from undiluted urine samples by different mixtures of carbon tetrachloride ($CCl_4$) and dichloromethane ($CH_2Cl_2$). Sample size: 10 ml urine; tube size: 50 ml syringe; amount of "Extrelut": 6 g; volume of each fraction: 1.4 ml.

Tab. 1. Reproducibility of fractionation using the systems outlined in fig. 2a (n = 10). Temperature was 25 °C.

| Steroid | Recovery in Fraction I Mean ± SD (%) | Recovery in Fraction II Mean ± SD (%) |
|---|---|---|
| Deoxycortisol | 95.02 ± 4.10 | 6.62 ± 3.19 |
| Cortisol | 1.5 ± 0.47 | 94.4 ± 3.35 |
| Oestradiol | 97.4 ± 0.35 | 2.5 ± 0.29 |
| Oestriol | 8.6 ± 1.17 | 91.3 ± 1.16 |

2) magnitude and reproducibility of recovery is better;

3) the procedure is faster and more convenient;

4) if using the home-made tubes described here instead of the ready-for-use ones offered commercially (6), total costs are lower;

5) the high efficiency of fractionation promotes this technique as a highly practicable technique for separating steroids interfering antigenically in immunological assays. For example, this latter quality is well demonstrated for the isolation of deoxycortisol and cortisol (fig. 2a) necessary in the metyrapone test (7) or for the isolation of oestrogens (fig. 2b) in the analytical field of reproductive endocrinology (8).

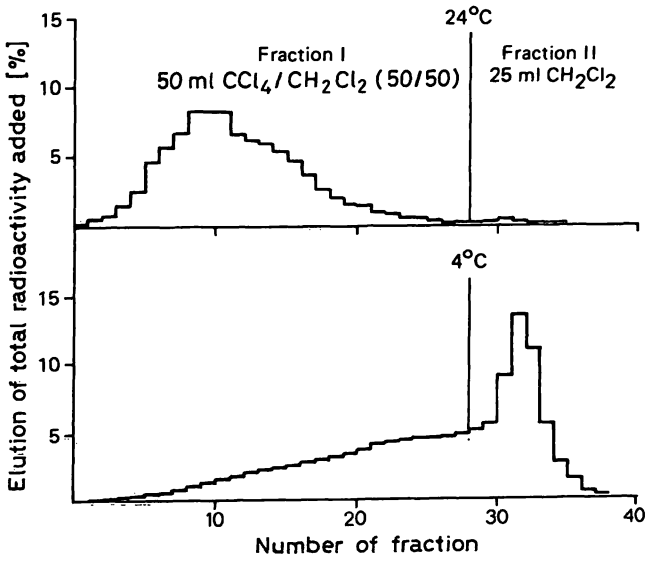


Fig. 3. Influence of temperature on fractionation profile of deoxycortisol from urine. Conditions were as outlined in fig. 2a.

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